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खाद्य रंगों के लिए नमूने लेने और परीक्षण की पद्धतियाँ

(दूसरा पुनरीक्षण)

Indian Standard

METHODS OF SAMPLING AND TEST FOR FOOD COLOURS

(Second Revision)

Second Reprint SEPTEMBER 2007
(Including Amendment No 1)

UDC 664.099 62 : 620.113

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BUREAU OF INDIAN STANDARDS
MANAK BHAVAN, 9 BAHADUR SHAH ZAFAR MARG
NEW DELHI 110002

February 1995 **Price Group 8**

AMENDMENT NO. 1 OCTOBER 1996
TO
IS 1699 : 1995 METHODS OF SAMPLING AND TEST FOR FOOD COLOURS

(Second Revision)

(*First cover page and page* 1, *Title*) — Insert 'synthetic' between 'for' and 'food' and affect the same change wherever it appears in the standard.

(*Page* 17, *clause* **16.2.1**, *first sentence*) — Substitute the following for the existing:

'Take the quantity of the standard lead solution of concentration equivalent to the limits specified in the individual standard in 50-ml Nessler tube and add about 23 ml of water.'

(FAD 8)

Printed at Simco Printing Press, Delhi

Food Additives Sectional Committee, FAD 8

FOREWORD

This Indian Standard (Second Revision) was adopted by the Bureau of Indian Standards, after the draft finalized by the Food Additives Sectional Committee had been approved by the Food and Agriculture Division Council

These methods are intended to provide uniform procedure for sampling and testing of food colours permitted under the *Prevention of Food Adulteration Rules*, 1955, Ministry of Health and Family Welfare, Government of India This standard includes the general tests applicable to all the permitted food colours. The method for the determination of the total dye content by the spectrophotometric method or any specific method has been given in the relevant Indian Standard Specifications for food colours whereas the general titanium trichloride method has been prescribed in this standard

This standard was initially issued in two parts Part I in 1960 and Part II in 1963 The amalgamated revised version of 1974 *inter alia* incorporated column chromatography method for determination of dye intermediates had also been prescribed

This standard is once again revised to cover the methods for determination of leuco base in sulphonated triarylmethane colouring matters; chloride and sulphate, and heavy metals have been incorporated The instrumental method for determination of metallic impurities have also been incorporated.

In reporting the results of a test or analysis made in accordance with this standard, if the final value, observed or calculated, is to be rounded off, it shall be done in accordance with IS 2 : 1960 'Rules for rounding off numerical values (*revised*)'.

Indian Standard

METHODS OF SAMPLING AND TEST FOR FOOD COLOURS

(Second Revision)

1 SCOPE

This standard prescribes the methods of sampling and test for food colours.

2 REFERENCES

The following Indian Standards are necessary adjuncts to this standard:

IS No.	*Title*
265 : 1993	Hydrochloric acid (*fourth revision*)
1070 : 1992	Reagent grade water (*third revision*)
2088 : 1983	Methods for determination of arsenic (*second revision*)
2491 : 1972	Code for hygienic conditions for food processing units (*first revision*)
4905 : 1968	Methods for random sampling

3 QUALITY OF REAGENTS

Unless specified otherwise, pure chemicals and distilled water (*see* IS 1070 : 1992) shall be employed in tests.

NOTE — 'Pure chemicals' shall mean chemicals that do not contain impurities which affect the test results.

4 SAMPLING

4.1 General Requirements of Sampling

4.1.0 In drawing, preparing, storing and handling test samples, the precautions and directions as given below shall be observed.

4.1.1 The samples shall be taken in a protected place not exposed to damp air, dust or soot.

4.1.2 The sampling instrument shall be clean and dry.

4.1.3 Precautions shall be taken to protect the sample, the material being sampled, the sampling instrument and the containers for samples from adventitious contamination.

4.1.4 To draw a representative sample, the contents of each container selected for sampling shall be mixed as thoroughly as possible by suitable means.

4.1.5 The sample shall be placed in clean, dry, air-tight glass containers or other suitable containers on which the material has no action.

4.1.6 The sample containers shall be of such a size that they are almost completely filled by the sample.

4.1.7 Each sample container shall be sealed air-tight with a stopper after filling, and marked with full details of sampling, date of sampling, batch and code number.

4.1.8 The samples shall be stored in such a manner that the temperature of the material does not vary unduly from the normal atmospheric temperature.

4.1.9 The sampling shall be done by a person agreed to between the purchaser and the supplier and in a presence of the purchaser (or his representative) and the supplier (or his representative).

4.2 Scale of Sampling

4.2.1 *Lot*

All the containers of the same material produced under the same conditions of manufacture shall be grouped together to constitute a lot.

4.2.1.1 The sample shall be tested from each lot for ascertaining the conformity of the material to the requirements of the specification.

4.2.2 The number of containers to be selected from the lot shall depend on the size of the lot and shall be in accordance with col 1 and 2 of Table 1.

Table 1 Number of Containers to be Selected for Sampling

Lot Size (1)	No. of Containers to be Selected (2)
2 to 15	2
16 to 50	3
51 to 150	5
151 and above	8

4.2.3 These containers shall be selected at random from the lot. For this purpose, reference may be made to IS 4905 : 1968.

4.3 Test Samples and Referee Sample

Draw with an appropriate sampling instrument, small quantities of the material from different parts of each container selected according to col 2 of Table 1. Mix all the portions so drawn, thoroughly, to form a composite sample weighing not less than 30 g. Divide the composite sample into three equal parts to form test samples. Each part thus obtained shall constitute the test sample weighing not less than 10 g and shall be sufficient to conduct all the tests. The test samples shall be transferred immediately to thoroughly clean and dry containers which shall be sealed air-tight. These shall be labelled with the particulars given in **4.1.7**. One sample shall be for the purchaser, the second for the supplier and the third sample bearing the seals of the purchaser and the supplier shall constitute the referee sample to be used in case of dispute between the purchaser and the supplier and shall be kept at a place agreed to between the purchaser and the supplier.

4.4 Criterion for Conformity

4.4.1 The test for the various characteristics shall be performed on the composite samples and shall meet the corresponding requirements specified in the standard for that particular food colour.

4.4.2 In case any of the samples selected as per col 2 of Table 1 fail in any of the tests, two more samples shall be selected from the unopened containers from the sample lot for retesting. If these two samples satisfy the requirements of the specification, the lot shall be deemed to comply with the requirements specified in the standard for that particular food colour. If either of the two samples fail in the retests, the lot shall be deemed as not conforming to the requirements specified in the standard for that particular food colour.

5 DETERMINATION OF TOTAL DYE CONTENT (TITANIUM TRICHLORIDE METHOD)

5.1 Reagents

5.1.1 *Sodium Citrate*

5.1.2 *Standard Potassium Dichromate Solution* — 0.1 N.

5.1.3 *Standard Titanium Trichloride Solution* — 0.1 N, prepared and standardized as described in **5.1.3.1** and **5.1.3.2**.

5.1.3.1 Prepare a 15 percent (*m/v*) solution of titanium trichloride. Take 200 ml of this solution, add 150 ml of concentrated hydrochloric acid (sp gr 1.16) and dilute to 2 000 ml so as to make the solution approximately 0.1 N. Place the solution in a container provided with an arrangement to maintain it in an atmosphere of hydrogen and allow to stand for two days for absorption of residual oxygen.

5.1.3.2 *Standardization of titanium trichloride solution*

Weigh 3 g of ferrous ammonium sulphate [$FeSO_4(NH_4)_2SO_4.6H_2O$] and transfer to a 500-ml flask. Introduce a stream of carbon dioxide and add 50 ml of freshly boiled water and 25 ml of sulphuric acid (40 percent *m/v*). Then without interrupting the current of carbon dioxide, add rapidly 40 ml of the standard potassium dichromate solution. Add the titanium trichloride solution until the calculated end-point is nearly reached. Then add quickly 5 g of ammonium thiocyanate (NH_4CNS) and complete the titration. Run a blank on 3 g of the ferrous ammonium sulphate using the same quantities of water, sulphuric acid, ammonium thiocyanate and the current of carbon dioxide. From the net volume of titanium trichloride, calculate the normality of titanium trichloride as follows:

$$\text{Normality of } TiCl_3 = \frac{\text{ml of } K_2Cr_2O_7 \times \text{normality of } K_2Cr_2O_7}{\text{ml of } TiCl_3}$$

5.2 Procedure

Prepare 1.0 percent (*m/v*) aqueous solution of the material. Take a quantity of the solution corresponding to about 20 ml of the standard titanium trichloride solution in a 500-ml Erlenmeyer flask. Add 15 g of sodium citrate and dilute with water to a volume of 150 to 200 ml. Heat to boil and titrate with the standard titanium trichloride solution till colourless. Calculate percentage of pure dye in the material.

1 ml of 0.1 N $TiCl_3$ = 0.013 36 g of Tartrazine
0.011 31 g of Sunset Yellow
0.023 32 g of Indigo Carmine
0.015 11 g of Ponceau 4 R
0.012 56 g of Carmoisine
0.039 64 g of Brilliant Blue FCF
0.040 44 g of Fast Green FCF

6 DETERMINATION OF LOSS ON DRYING

6.1 Procedure

Weigh accurately about 2 g of the material in a tared weighing bottle fitted with a ground-glass lid. A weighing bottle of squat form about 50 mm in diameter and 30 mm in height is suitable. Heat for 3 hours in an air-oven at 135 ± 2°C, cool in a desiccator and weigh.

6.2 Calculation

$$\text{Loss on drying, percent by mass} = \frac{100\,(M_1 - M_2)}{M_1 - M}$$

where

M_1 = mass, in g, of the weighing bottle with the material before heating;

M_2 = mass, in g, of the weighing bottle after heating; and

M = mass, in g, of the weighing bottle.

7 DETERMINATION OF WATER-INSOLUBLE MATTER

7.1 Apparatus

7.1.1 *Prepared Gooch Crucible*

Digest a good grade retentive asbestos with dilute hydrochloric acid (1 : 3), wash free from acid and decant to remove fine particles. Prepare well-packed asbestos mat of suitable thickness in a Gooch, wash with hot water, dry, ignite, rewash, redry at 135°C, cool in a desiccator and weigh. Repeat washing, heating and drying to constant mass. (Alternatively, sintered glass filter, Grade 4, may be used).

7.2 Procedure

Dissolve 4.5 to 5.5 g of the material in 200 ml of hot water (80-90°C) and allow the solution to cool to room temperature. Filter through the tared Gooch or sintered glass filter, wash with cold water until the washings are colourless. Dry at 135 ± 2°C for 3 hours. Cool in a desiccator and weigh.

7.3 Calculation

$$\text{Water-insoluble matter, percent by mass} = \frac{100\,(M_2 - M_1)}{M}$$

where

M_1 = mass, in g, of the prepared Gooch;

M_2 = mass, in g, of the Gooch with the residue; and

M = mass, in g, of the material taken for the test.

8 DETERMINATION OF COMBINED EXTRACTS

8.1 Apparatus

Separator or continuous extractor of 250 ml capacity.

8.2 Reagents

8.2.1 *Isopropyl Ether*

Wash one litre of *isopropyl* ether with (a) two 100-ml portions of sodium hydroxide (0.5 N), (b) saturated solution of ferrous sulphate, and (c) with three 100-ml portions of water.

8.2.2 *Sodium Hydroxide Solution* — 10 percent (*m*/*v*).

8.2.3 *Sodium Hydroxide Wash Solution* — 0.1 N.

8.2.4 *Dilute Hydrochloric Acid* (1 : 1)

Prepared by diluting hydrochloric acid, sp gr 1.16 (*see* IS 265 : 1993) with equal volume of water.

8.2.5 *Hydrochloric Acid Wash Solution*

Concentrated hydrochloric acid diluted 200 times.

8.3 Procedure for Extraction in the Separator

8.3.1 *Neutral Ether Extract*

Place the aqueous solution containing 10 g of the material in a separator and dilute to 200 ml. Extract with two 100-ml portions of the washed ether, shaking for one minute during each extraction. Decant ether into another clean separator and rinse the first separator with 10 ml of the ether, decanting into the second separator. Reserve the aqueous colour solution (*see* **8.3.2**). Wash combined extracts with 20 ml portions of water until the washings are colourless. Decant the ether into a beaker. Place the beaker on a water-bath or steam-bath in dust free atmosphere and allow the ether to evaporate to a volume of 50 ml. Transfer to a previously weighed evaporating dish of 250-ml capacity. Rinse the beaker with 40 ml of ether and drain into the same dish. Evaporate the remaining ether, dry in a desiccator and weigh. Repeat the process of evaporating, drying and weighing till the difference between two successive weighings is less than a milligram. Note the lowest mass. Calculate the mass of the neutral ether extract.

NOTE — Do not fill the beaker or dish more than one-third of the capacity and do not allow the ether to boil.

8.3.2 *Alkaline Ether Extract*

To the reserved aqueous solution (*see* **8.3.1**), add 2 ml of sodium hydrochloride solution and proceed in the same manner as in **8.3.1** except to wash the ether extract with sodium hydroxide wash solution instead of water. Reserve the aqueous solution for use in **8.3.3**. Calculate the mass of the alkaline ether extract.

8.3.3 *Acid Ether Extract*

To the reserved aqueous solution (*see* **8.3.2**) add 3 ml of dilute hydrochloric acid (1 : 1) and proceed in the same manner as in **8.3.1** except to wash the ether extract with hydrochloric acid wash solution instead of water. Discard the colour solution. Calculate the mass of the acid ether extract.

8.4 Procedure for Extraction in the Continuous Extractor

8.4.1 *Neutral Ether Extract*

Dissolve 5 g of the material in water and extract in the continuous extractor with about 100 ml of the ether for 5 hours. Transfer the extract to the separator, rinse the flask with 10 ml of ether and add the rinsings to the main extract. Proceed in the same manner as in **8.3.1** from the washing stage onwards.

8.4.2 *Alkaline Ether Extract*

To the aqueous solution in the extractor add 2 ml of sodium hydroxide solution and proceed in the same manner as in **8.4.1** except to wash the ether extract with sodium hydroxide wash solution instead of water.

8.4.3 *Acid Ether Extract*

Add 3 ml of dilute hydrochloric acid solution to the alkaline aqueous solution of the material in the extractor and proceed in the same manner as in **8.4.1** except to wash the ether extract with hydrochloric acid wash solution instead of water.

9 SUBSIDIARY DYES

9.1 Principle

The subsidiary dyes are separated from the main dye by ascending paper chromatography and are extracted separately from the paper The optical densities of the extracts are measured at their wavelengths of maximum absorption in the visible spectrum and are used to calculate the content of subsidiary dyes as a percentage by mass of the sample

9.2 Apparatus

9.2.1 *Chromatography Tank and Ancillary Equipment*

Suitable apparatus is shown in Fig. 1 and comprising the following.

a) a glass tank (*A*) and glass cover (*B*);

b) a supporting frame (*C*) for the chromatography grade paper sheets;

c) a tray (*D*) for developing solvent;

d) a secondary frame (*E*) supporting drapes of filter paper; and

e) sheets of chromatography grade paper, not less than 200mm × 200 mm (Whatman No. 1 Chromatography grade is suitable).

9.2.2 *Microsyringe*

Capable of delivering 0.1 ml with a tolerance of ±0.002 ml.

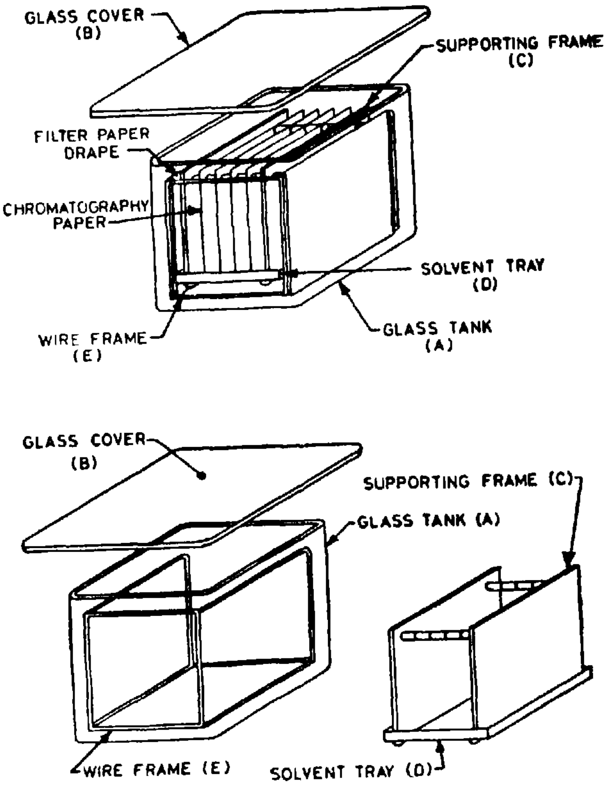


Fig 1 Chromatography Apparatus

9.2.3 *Spectrophotometer*

9.3 Reagents

9.3.1 *Chromatography Solvents*

1. Water : ammonia (sp gr 0.880): trisodium citrate (95 ml : 5 ml : 2 g)
2. *n*-butanol : water : ethanol : ammonia (sp gr 0.880) (600 : 264 : 135 : 6)
3. Butan-2-one : acetone : water (7 : 3 : 3)
4. Butan-2-one : acetone : water : ammonia (sp gr 0.880) (700 : 300 : 300 : 2)
5. Butan-2-one : acetone : water : ammonia (sp gr 0.880) (700 : 160 : 300 : 2)
6. *n*-butanol : glacial acetic acid : water (4 : 1 : 5)

Shake for 2 minutes, allow layers to separate. Use the upper layer as the chromatography solvent. The particular solvent to be used as given in individual specifications.

9.3.2 *Extracting Solvent*

A mixture of equal volumes of acetone and water.

9.3.3 *Sodium Bicarbonate* — 0.05 N.

9.4 Procedure

9.4.1 Not less than 2 hours before carrying out the determination, arrange the filter-paper drapes in the glass tank and pour over the drapes and into the bottom of the tank sufficient of the atmosphere saturating solvent to cover the bottom of the tank to a depth of approximately 1 cm. Place the solvent tray *D* in position and fit the cover to the tank.

9.4.2 Mark out a sheet of chromatography grade paper as shown in Fig. 2. Apply 0.10 ml of a 1.0 percent aqueous solution of the dye as uniformly as possible within the confines of the 180 mm × 7 mm rectangle, holding the nozzle of the microsyringe steadily in contact with the paper. Allow the paper to dry at room temperature for 1 to 2 hours, or at 50°C for 5 minutes followed by 15 minutes at room temperature. Mount the sheet, together with a plain sheet to act as a blank, in frame *C*. Pour sufficient of the chromatography solvent into the tray *D* to bring the surface of the solvent about 1 cm below the base line of the sheet of chromatography paper. The volume necessary will depend on the dimensions of the apparatus and should be predetermined. Put the frame *C* into position and replace the cover. Allow the solvent front to ascent the full height of paper, development being continued for 1 hour afterwards, then remove the frame *C* and transfer it to a drying cabinet at 50°C to 60°C for 10 to 15 minutes. Remove the sheets from frame *C*.

NOTE — If required, several chromatograms may be developed simultaneously.

9.4.3 Cut each subsidiary band from the sheet as a strip, and cut an equivalent strip from the corresponding position of the plain sheet. Place each strip, subdivided into a suitable number of approximately equal portions, in a separate test-tube. Add 5.0 ml of extracting solvent to each test-tube, swirl for 2 to 3 minutes, add 15.0 ml of the sodium bicarbonate solution and shake the tube to ensure mixing. Filter the coloured extracts and blanks through a 9-cm filter paper of open texture and determine wavelengths of maximum absorption, using cells of suitable light path, against a filtered mixture of 5.0 ml of extracting solvent and 15.0 ml of the sodium bicarbonate solution. Measure the optical densities of the extract of the blank strips at the wavelengths at which those of the corresponding coloured extracts were measured.

9.5 Calculation

The content of the subsidiary dye, expressed as a

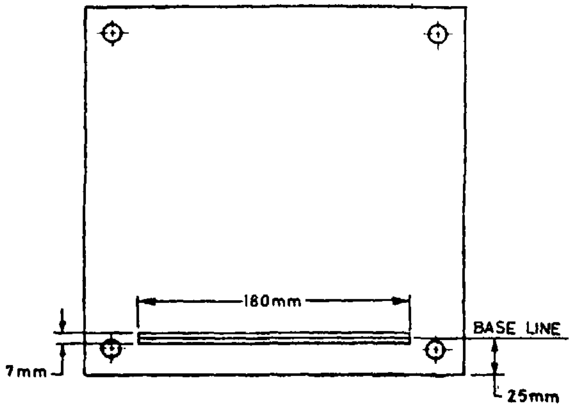


FIG. 2 METHOD OF MARKING OUT CHROMATOGRAPHY PAPER

percentage (S) of the sample, is given by:

$$S = F\ [(D_1 + D_2 + ...) - (b_1 + b_2\ ....)]$$

where

F is the mean conversion factor and is equal to 11.4;

D_1, D_2 etc, are the optical densities of the subsidiary dye extracts; and

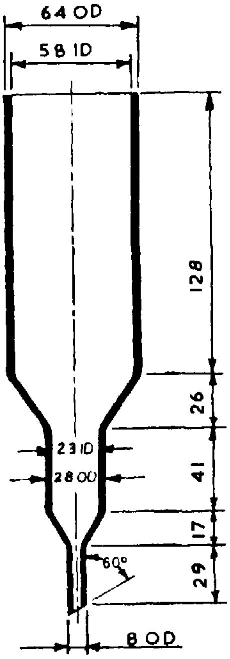
b_1, b_2, etc, are the optical densities of the extracts of the corresponding blanks.

The conversion factor F in the above expression is derived from the extraction coefficient of the main colour, not that of the subsidiaries, and from the other constants of the determination.

10 DETERMINATION OF DYE INTERMEDIATES (ORGANIC COMPOUNDS OTHER THAN COLOURING MATTERS)

10.1 Apparatus

10.1.1 *Chromatographic Tube* — *See* Fig. 3.



All dimensions in millimetres

FIG. 3 CHROMATOGRAPHIC TUBE

10.1.2 *Suitable Spectrophotometer for Use in the Ultra-Violet Range*

10.1.3 *Column Preparation*

Prepare a slurry of Whatman powdered cellulose (or equivalent) in a 25 percent ammonium sulphate (very low in iron) solution. If other cellulose is used, the iron content should be very low. Prepare the column and pass 200 ml of 25 percent ammonium sulphate solution through it. The ultra-violet absorption of the solution shall be sufficiently low to avoid interference with the intended analysis. Use about 75 g of cellulose to 500 ml of liquid. Place a small disc of stainless steel gauze in the constriction above the tip of the tube. Pour sufficient slurry into the tube to give a column to a height of about 5 cm in the mouth of the tube. Tap the tube occasionally to ensure a well-packed column. Wash the column with 200 ml of the eluent.

10.2 Procedure

10.2.1 Place 0.200 g of the dye sample in a beaker and dissolve in 20 ml of water. Add approximately 5 g of powdered cellulose. Add 50 g of ammonium sulphate to the dye. Transfer the mixture to the column, rinse the beaker with 25 percent ammonium sulphate solution and add washings to the tube. Allow the column to drain until flow ceases, or nearly so. Add the ammonium sulphate solution to the column at a rate equivalent to the rate of flow through the column. Collect the effluent in 100 ml fractions. Continue until 12 fractions have been collected. Reserve the column and contents until the last fractions have been examined. Mix each fraction well, and obtain the ultra-violet absorption spectra of each solution from 220 to 400 nm. The specific spectra may be choosen depending on the nature of the dyes. If the spectrum of the twelfth fraction shows the presence of any intermediate, continue collecting fractions until the intermediates present are eluted. Usually only one intermediate is encountered. Identification and quantitative determination shall be accomplished by comparison of the absorption spectra of the eluted material with the spectra of solutions of the pure intermediates in the same solvent. When more than one intermediate is present in significant quantities in any fractions, the spectrophotometric data shall indicate this. In such cases, the amounts of the various intermediates should be determined by the procedure customarily used in spectrophotometric analysis of mixtures of absorbing materials.

10.2.2 Some samples contain small amounts of various materials, particularly inorganic salts, that contribute 'background absorption'. Correction for this is made as follows:

> Determine the amount of such absorption of the fraction collected from the column immediately before and after the fraction immediately following those fractions in which the intermediates are encountered. Subtract one-half of the sum of these two determinations from the observed absorbance of the fractions containing the intermediates. The remainder should be taken as the absorbance due to the intermediate present.

11 DETERMINATION OF UNSULPHONATED PRIMARY AROMATIC AMINES

11.1 Principle

Unsulphonated primary aromatic amines are extracted into toluene from an alkaline solution of the sample, re-extracted into acid and then determined spectrophotometrically after diazotisation and coupling. They are expressed as aniline unless they are known to be some other amine.

11.2 Apparatus

Visible range spectrophotometer.

11.3 Reagents

The reagents shall be of a recognized analytical reagent quality. Distilled water or water of at least equal purity shall be used.

11.3.1 *Toluene*

11.3.2 *Hydrochloric Acid* — 1 N solution (approx).

11.3.3 *Hydrochloric Acid* — 3 N solution (approx).

11.3.4 *Potassium Bromide* — 50% solution (approx).

11.3.5 *Sodium Carbonate* — 2 N solution (approx).

11.3.6 *Sodium Hydroxide* — 1 N solution (approx).

11.3.7 *Sodium Hydroxide* — 0.1 N solution (approx).

11.3.8 *R Salt* (*2-naphthol-3 : 6-disulfonic acid, disodium salt*) — 0.05 N solution (approx).

11.3.9 *Sodium Nitrite* — 0.5 N solution (approx).

11.3.10 *Standard Aniline Solution*

Solution A — Into a small weighing beaker, weigh 0.100 g of redistilled aniline, then wash it into a 100-ml one-mark volumetric flask, rinsing the beaker several times with water. Add 30 ml of approx 3 N hydrochloric acid solution and dilute to the mark with water at room temperature.

Solution B — Dilute 10.0 ml of solution A with water to 100 ml in a one-mark volumetric flask and mix well. 1 ml of this solution will be equivalent to 0.000 01 g of aniline.

NOTE — Prepare solution B freshly when required

11.4 Procedure

11.4.1 *Preparation of Calibration Graph*

Measure 5, 10, 15, 20 and 25 ml of standard aniline solution B into a series of 100-ml one-mark volumetric flasks.

Dilute to 100 ml with approx 1N hydrochloric acid solution and mix well. Pipette 10 ml of each mixture into clean, dry test tubes and cool for 10 minutes by immersion in a beaker of ice/water mixture. To each tube add 1 ml of the potassium bromide solution and 0.05 ml of the sodium nitrite solution. Mix and allow to stand for 10 minutes in the ice/water bath. Into each of the five 25-ml volumetric flasks, measure 1 ml of the R salt solution, and 10 ml of the sodium carbonate solution. Pour each diazotised aniline solution into a separate flask containing R salt solution, rinsing the test tubes with a few drops of water. Dilute to the mark with water, stopper the flasks, mix the contents well and allow to stand for 15 minutes in the dark. Measure the absorbance of each coupled solution at 510 nm in 1 cm cells, using as a reference a mixture of 10.0 ml of 1N hydrochloric acid solution, 10.0 ml of the sodium carbonate solution, and 2.0 ml of the R salt solution, mass of aniline in each 100 ml of aniline solution.

11.4.2 *Preparation and Examination of Test Solution*

Weigh, to the nearest 0.01 g, about 2.0 g of the colour sample into a separating funnel containing 100 ml of water, swirl down the sides of the funnel with further 50 ml of water. Swirl to dissolve the sample, and add 5 ml of 1N sodium hydroxide solution. Extract with two 50-ml portions of 0.1N sodium hydroxide solution to remove traces of colour. Extract the washed toluene with three 10 ml portions of 3 N hydrochloric acid solution and diltue the combined extract to 100 ml with water. Mix well. Call this solution T.

Pipette 10.0 ml of solution T into a clean, dry test tube, cool for 10 minutes by immersion in a beaker of ice/water mixture, add 1 ml of the potassium bromide solution and proceed as described above for the preparation of the calibration graph, starting with the addition of 0.05 ml of the sodium nitrite solution.

Use as the reference solution in the measurement of absorbance, a solution prepared from 10.0 ml of the test solution, 10 ml of the sodium carbonate solution and 2.0 ml of the solution, diluted to 25.0 ml with water.

Read from the calibration graph the mass of aniline corresponding to the observed optical density of the test solution.

11.5 Calculation

$$\text{Percentage of unsulphonated primary aromatic amine (as aniline) in sample} = \frac{\text{mass of aniline} \times 100}{\text{mass of sample taken}}$$

12 DETERMINATION OF LEUCO BASE IN SULPHONATED TRIARYLMETHANE COLOURING MATTER

12.1 Principle

Air is blown through an aqueous solution containing the chloride and dimethylformamide. Under these conditions the leuco base is oxidized to colouring matters and the increase in absorptivity is a measure of the amount of leuco base originally present.

12.2 Reagents

12.2.1 *Dimethylformamide (DMF)*

Solution A — Weigh 10.0 g of $CuCl_2.2H_2O$ and dissolve in 200 ml of DMF. Transfer to a 1-litre volumetric flask and make up to the mark with DMF.

Solution B — Accurately weigh the specified quantity of sample, dissolve in approximately 100 ml water, transfer quantitatively to a 10 litre volumetric flask and make up to the mark with water.

12.3 Procedure

12.3.1 Prepare the following solutions

Solution A — Pipette 50 ml DMF into a 250-ml volumetric flask. Cover with parafilm and place in the dark.

Solution B — Accurately pipette 10 ml of solution B into a 250-ml volumetric flask. Add 50 ml DMF. Cover with parafilm and place in the dark.

Solution C — Pipette 50 ml of solution A into a 250-ml volumetric flask. Bubble air through this solution for 30 minutes in the following manner.

Insert a 5 ml pipette into a box attached to a bench air flow source. Turn on the air, slowly. Stick the pipette down into the solution in the flask and adjust the air flow to a rapid but controlled rate. After 30 minutes pull the pipette out of the solution and rinse the sides of the pipette into the flask with water from a wash bottle. Then turn off the air flow.

Solution D — Accurately pipette 10 ml solution B into two separate 250-ml volumetric flasks in the same manner as used for solution B. Add 50 ml solution A to each flask. Bubble air through the solutions for 30 minutes, using the above method.

12.3.2 After 30 minutes of rapid bubbling of air through the solutions, dilute all 5 flasks nearly to volume with water. Heat is evolved when DMF and water are mixed, so place the flask in a water bath of tap water until they have cooled to room temperature. Do not leave them for longer than necessary; 5-10 minutes is normally long enough. Bring accurately to volume with water. Run the solutions on the spectrophotometer immediately. The entire procedure should be completed as quickly as possible.

12.4 Spectrophotometric Determination

12.4.1 Draw the following curves from 700-500 nm using an absorbance range of 0.1 and 1 cm cells. Run all curves on the same spectrogram, and (for maximum accuracy) take readings for the numerical display at the maximum between 620 and 635 nm by cranking back after the curve is drawn.

12.4.2 *Reference Sample*

Curve	*Cell*	*Cell*	*Comments*
1	a	a	Set zero at 700 nm, run curve; record absorbance at Abs std for colouring matter
2	a	b	Run curve without readjusting zero setting; record absorbance at maximum
3	c	c	Set zero at 700 nm; record absorbance at Abs std for colouring matter
4a	c	d_1	Run curve without readjusting zero setting; record absorbance at maximum
4b	c	d_2	Run curve without readjusting zero setting; record absorbance at maximum
			(d_1 and d_2 are duplicate determinations)

NOTE — Cells must be thoroughly rinsed before each run. For the flow through cell, use 3 separate rinses of at least 40 ml of the sample solution to be run.

12.5 Calculation

$$\text{Leuco base, percent by mass} = \frac{[(4-3)-(2-1)] \times 25 \times 100\%}{a \times 1 \text{ cm} \times M \times \text{ratio}}$$

where

a = absorptivity of 100% colouring matters;

M = mass, in mg, of sample taken for test; and

$$\text{ratio} = \frac{\text{MW of colouring matter}}{\text{MW of leuco base}}$$

13 DETERMINATION OF CHLORIDE AS SODIUM CHLORIDE

13.1 Apparatus

Potentiometric titration apparatus, with silver indicator electrode, calomel reference electrode, and saturated potassium sulphate bridge.

13.2 Procedure

Accurately weigh 0.5-1.0 g of the dye sample, dissolve in 100 ml of water, and acidify with 5 ml of 1.5 N nitric acid solution. Place the silver electrode in the colour solution and connect the calomel electrode to the solution by means of the saturated potassium sulphate bridge. The saturated potassium sulphate bridge may be eliminated by using a glass electrode as the reference electrode; this simplifies the apparatus considerably, and the glass electrode is sufficiently constant to be used as a reference for this type of titration.

Determine the chloride content of the solution by titration against the 0.1 N silver nitrate solution ($AgNO_3$) and calculate the result as sodium chloride. [1 ml of 0.1 N silver nitrate solution = 0.005 85 g of sodium chloride ($NaCl$)].

Express the result as a percentage of the mass of sample taken.

14 DETERMINATION OF SULPHATE AS SODIUM SULPHATE

Accurately weigh about 5.0 g of the sample, transfer it to a 250 ml conical flask and dissolve in about 100 ml of water by heating on a water bath. Add 35 g of sulphate-free sodium chloride, stopper the flask, and swirl at frequent intervals during 1 hour. Cool, transfer with saturated sodium chloride solution to a 250 ml measuring flask and dilute to the mark at 20°C. Shake the flask, and filter the solution through a dry filter paper. Pipette 100 ml of the filtrate into a 500 ml beaker, dilute to 300 ml with water and acidify with hydrochloric acid, adding 1 ml in excess. Heat the solution to boiling, and add an excess of 0.25 N barium chloride solution, drop by drop, with stirring. Allow the mixture to stand on a hot plate for 4 hours, or leave it overnight at room temperature and then bring it to about 80°C and allow the precipitate to settle. Filter off the precipitated barium sulphate, wash with hot water, and ignite at a dull red heat in a tared crucible until a constant mass is obtained.

Carry out a blank determination, apply any necessary correction to the mass of barium sulphate found in the test, and calculate the result as sodium sulphate.

$$\text{Mass of sodium sulphate } (Na_2SO_4) \text{ in sample} = 2.5 \times \text{corrected mass of barium sulphate } (BaSO_4) \times 0.608\,6$$

Express the result as a percentage of the mass of sample taken.

15 DETERMINATION OF METALLIC IMPURITIES

15.0 Both instrumental and chemical methods have been given for determination of metallic impurities. For certain metallic impurities, namely; antimony, barium, cadmium, zinc and mercury only instrumental methods have been given. For lead, arsenic, copper and chromium both instrumental and chemical methods have been given. In cases where more than one method has been given, any of these may be used. However, in case of dispute instrumental method shall be used as referee method.

15.1 Instrumental Methods

The instrumental methods described in this section may be employed in the quantitative analysis of certain metallic impurities in food additives.

Method I is applicable to substances soluble in dilute acids or mixtures of acids. Method II is used for other substances.

15.1.1 *Principle*

The samples are dissolved in acid or digested in a mixture of sulphuric, nitric and in some cases perchloric acids. The barium, cadmium, lead, copper, chromium, and zinc in solution are determined by conventional flame atomic absorption spectroscopy. Antimony and arsenic are determined by using a hydride generation technique. Alternatively, antimony may be determined by flame atomic absorption but the hydride generation technique is more sensitive.

15.1.2 *General Precautions*

Because of the minute amounts of metals involved, special care shall be taken to reduce the reagent blanks to as low a value as possible and to avoid contamination during the test. All apparatus should be thoroughly cleaned with a mixture of hot dilute acids (1 part hydrochloric acid, 1 part concentrated nitric acid, and 3 parts water) followed by thorough washing with water immediately before use.

15.1.3 *Apparatus*

15.1.3.1 *Kjeldahl flasks* — of silica or borosilicate glass fitted with an extension to the neck by means of a B24 ground joint, as shown in Fig. 4. The extension serves to condense the fumes and carries a tap funnel through which the reagents are introduced.

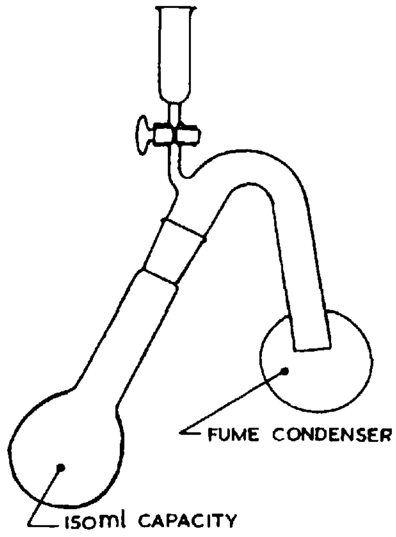


Fig 4 Modified Kjeldahl Flask (Open Type)

15.1.3.2 *Atomic absorption spectrophotometer* — Any instrument operating in the absorption mode may be used providing it has facilities for the selection of the required oxidant/fuel combination from a choice of air, argon, nitrous oxide, hydrogen and acetylene and has a wavelength range from 180 to 600 nm.

A hydride generation vessel accessory is also required and is available from all the major commercial manufacturers of atomic absorption equipment. For operations in emission mode and measurements of absorption involving the generation of a gaseous hydride, a potentiometric recorder is necessary, preferably a multi-range type covering the range 1-20 mV.

15.1.4. *Reagents*

15.1.4.0 Reagents shall be of an order of purity higher than accepted analytical reagent grade quality. Metal-free water (see below) shall be used throughout:

a) *Nitric acid* — sp gr 1.42.

b) *Perchloric acid* — 60% (*m/m*) solution.

c) *Sulphuric acid* — 98%.

d) *Hydrochloric acid* — sp gr 1.16 to 1.18.

e) *Hydrochloric acid* — 5 N solution prepared by dilution of reagent (d) with metal-free distilled water.

f) *Water* — metal-free. Distilled water may be re-distilled from an all-glass apparatus or may be passed down a column of cation exchange resin, for example, Amberlite IR 120 (H).

g) *Sodium sulphate*

h) *Sodium borohydride pellets*

j) *Potassium chloride*

15.1.4.1 *Standards*

Use commercially available standard solutions or prepare solutions as follows:

a) *Standard copper solution* — Dissolve 3.928 g of pure copper sulphate $CuSO_4.5H_2O$ in water, dilute to 1 000 ml at 20°C with water in a one-mark graduated flask. Dilute 10 ml to 100 ml with water in a one-mark graduated flask as required. 1 ml contains 100 µg Cu.

b) *Standard zinc solution* — Dissolve 1.000 g of pure zinc powder in a mixture of 10 ml water and 5 ml hydrochloric acid [special reagent (d)] and dilute to 1 000 ml at 20°C with water, in a one-mark graduated flask. Dilute 10 ml to 100 ml with water in a one-mark graduated flask as required. 1 ml contains 100 µg Zn.

c) *Standard chromium solution* — Dilute 5.80 ml of 0.1 N potassium dichromate solution to 100 ml at 20°C with water in a one-mark graduated flask as required. 1 ml contains 100 µg Cr.

d) *Standard antimony solution* — Dissolve 2.668 g potassium antimony tartrate $K(SbO)C_4H_4O_6$ in distilled water, dilute to 1 000 ml at 20°C with water in a one-mark graduated flask. Dilute 10.0 ml to 100 ml with distilled water in a one-mark graduated flask as required. 1 ml contains 100 µg Sb.

e) *Standard lead solution* — Dissolve 1.60 g of lead nitrate, $Pb(NO_3)_2$ in nitric acid (10 ml of concentrated nitric acid diluted with 20 ml water, boiled to remove nitrous fumes, and cooled) and dilute to 1 000 ml with water in a one-mark graduated flask. Dilute 10.0 ml of this solution to 500 ml at 20°C with water in a one-mark graduated flask as required. 1 ml contains 20 µg Pb.

f) *Standard barium solution* — Dissolve 1.779 g barium chloride $BaCl_2.2H_2O$ in distilled water, dilute to 1 000 ml at 20°C with water in a one-mark graduated flask. Dilute 10.0 ml to 100 ml with water in a one-mark graduated flask as required. 1 ml contains 100 µg Ba.

g) *Standard arsenic solution* — Dissolve 1.320 g of arsenous oxide, As_2O_3 by warming at a temperature not exceeding 60°C with 14 ml of 5 N sodium hydroxide solution in a 100 ml beaker. Cool, add 0.2 ml of phenolphthalein indicator and neutralize with 6 N sulphuric acid. Transfer the solution to a 1 000 ml one-mark graduated flask containing 10 g of sodium hydrogen carbonate dissolved in water, washing out the beaker with water. Dilute to the mark with water at 20°C and mix. Dilute 5 ml of this solution to 1 000 ml at 20°C with water in a one-mark graduated flask as required. 1 ml contains 5 µg As.

h) *Standard cadmium solution* — Dissolve 2.282 g $3CdSO_4.8H_2O$ in distilled water, dilute to 1 000 ml at 20°C with water in a one-mark graduated flask. Dilute 10.0 ml of this solution to 500 ml at 20°C with water in a one-mark graduated flask. 1 ml contains 20 µg Cd.

15.1.5 *Preparation of Test Solutions*

15.1.5.0 Prepare the test solution according to Method I in the case of substances soluble in dilute acids. Use Method II for other substances.

15.1.5.1 *Method I*

Accurately weigh about 2.5 g of the sample and dissolve in a mixture of 4 ml of sulphuric acid and 5 ml of hydrochloric acid. Transfer the solution to a 50 ml one-mark graduated flask. If barium is to be measured from the solution, add 0.095 4 g of potassium chloride. Dilute to the mark with water. Call this 'Solution A'.

15.1.5.2 *Method II*

Accurately weigh about 2.5 g of the sample into a

100-150 ml Kjeldahl flask, and add 5 ml of dilute nitric acid. As soon as any initial reaction subsides, heat gently until further vigorous reaction ceases and then cool. Add gradually 4 ml of concentrated sulphuric acid at such a rate as not to cause excessive frothing on heating (5-10 min are usually required) and then heat until the liquid darkens appreciably in colour, that is, begins to char.

Add concentrated nitric acid slowly in small portions, heating between additions until darkening again takes place. Do not heat so strongly that charring is excessive or loss of arsenic may occur; small but not excessive amount of free nitric acid should be present throughout. Continue this treatment until the solution is only pale yellow in colour and fails to darken in colour on prolonged heating. If the solution is still coloured run in 0.5 ml of the perchloric acid solution and a little concentrated nitric acid and heat for about 15 minutes, then add a further 0.5 ml of *the perchloric acid solution and heat for a few minutes* longer. Note the total amount of concentrated nitric acid used. Allow to cool somewhat and dilute with 10 ml of water. The solution should be quite colourless (if much iron is present it may be faintly yellow). Boil down gently, taking care to avoid bumping, until white fumes appear. Allow to cool, add a further 5 ml of water and again boil down gently to fuming. Finally, cool, add 10 ml of 5N hydrochloric acid and boil gently for a few minutes. Cool and transfer the solution to a 50 ml one-mark graduated flask washing out the Kjeldahl flask with small portions of water. Add the washings to the graduated flask and dilute to the mark with water. If barium is to be measured from the solution, add before dilution 0.095 4 g of potassium chloride, as an ionizing buffer to prevent ionization of barium. Call this 'Solution A'.

Prepare a reagent blank using the same quantities of reagents as used in the sample oxidation.

15.1.6 *Measurement of Antimony, Barium, Cadmium, Chromium, Copper, Lead and Zinc by Atomic Absorption*

15.1.6.1 *Preparation of calibration curve solutions*

To a series of 100 ml one-mark volumetric flasks, pipette 0, 1, 2, 3, 4 and 5 ml of the appropriate standard solution [standards (a) to (f) and (h) *see* **15.1.4.1**] and dilute to about 50 ml. Add 8 ml concentrated sulphuric acid [reagent (c)] and 10 ml concentrated hydrochloric acid [reagent (d)]. Shake to dissolve. In the case of barium [standard (f)], add 0.191 g of potassium chloride as an ionization buffer. When solution is complete, dilute to the mark with metal free water.

These solutions then contain 1.0, 2.0, 3.0, 4.0 and 5.0 µg per ml of either barium, copper, zinc, chromium or antimony respectively, or 0, 0.2, 0.4, 0.6, 0.8 and 1.0 µg per ml of cadmium or lead.

15.1.6.2 *Instrumental conditions*

Select the wavelength and gases to be used for the particular element under consideration from the table below:

Element	*Wavelength* (nm)	*Gases*
Antimony	217.6	Air/acetylene
Barium	553.6	Nitrous oxide/acetylene
Cadmium	228.8	Air/acetylene
Chromium	357.9	Nitrous oxide/acetylene
Copper	324.8	Air/acetylene
Lead	283.3	Air/acetylene
Zinc	213.9	Air/acetylene

The recommended settings for the various instrumental parameters vary from model to model, and certain parameters require optimization at the time of use to obtain the best results. Instruments should therefore be adjusted as described in the manufacturer's instructions using the type of flame and wavelength settings specified above.

15.1.6.3 *Procedure*

Set the atomic absorption spectrophotometer to the appropriate conditions. Aspirate the strongest standard containing the element to be determined and optimize the instrument settings to give full-scale or maximum deflection on the chart recorder. Measure the absorbances of the other standards and plot a graph showing the net absorbance against the concentration of the element in the standard solutions. Aspirate the solution A obtained from dissolution or the wet oxidation of the sample and the corresponding blank solution and determine the net absorbance. Using the graph prepared above, determine the concentration of the element in the sample solution:

$$\text{Element in the sample, mg/kg} = \frac{\text{Concentration of element } (\mu\text{g/ml}) \times 50}{\text{Mass of sample taken (g)}}$$

15.1.7 *Measurement of Arsenic and Antimony by Atomic Absorption Hydride Technique*

15.1.7.0 Arsenic and antimony are determined after preparation of their volatile hydrides which are collected either in the generation vessel itself or, in some designs, in a rubber balloon attached to the vessel. The gases are then expelled with argon into a hydrogen flame.

15.1.7.1 *Preparation of calibration curve solution*

Into a series of 100-ml one-mark volumetric flasks add from a burette, 0, 1, 2, 3, 4 and 5 ml of standard arsenic or antimony solution [Standards (g) and (d)] and dilute to about 50 ml with distilled water. Add 8 ml concentrated sulphuric acid [reagent (c)] and 10 ml hydrochloric acid [reagent (d)]. Shake to dissolve, and when solution is complete, dilute to the mark with distilled water.

15.1.7.2 *Instrumental conditions*

Using the atomic absorption spectrophotometer with the appropriate hollow cathode or electrodeless discharge lamp, select the wavelength for either arsenic (193.7 nm) or antimony (217.6 nm)

15.1.7.3 *Procedure*

Measure 5.0 ml of the strongest standard into the generation vessel, add 25 ml water and 2 ml 5 N hydrochloric acid [reagent (e)]. Stopper the vessel and expel any air as described in the maker's instructions, filling the apparatus with argon. Isolate the vessel from the atomizer using the by-pass valve. Remove the atomizer and then quickly add 1 pellet of sodium borohydride weighing approximately 0.2 g and replace the stopper. Ensure that all the joints are secure

When the reaction slows (20-30 sec) open the appropriate taps to allow argon to drive the generated hydride into the flame When the hydride has all been expelled as shown by the recorder trace, return the taps to their original position and empty the vessel.

Optimize the instrument settings to give full scale deflection for the strongest standard Measure the other standards, the sample and the blank solution using the same procedure.

Plot a graph relating peak height on the recorder to concentration of the arsenic or antimony in the standards Using the net absorbance of the sample, read from the graph the concentration of arsenic or antimony in the solution.

15.1.7.4 *Calculation*

$$\text{Arsenic or antimony in the sample, mg/kg} = \frac{\text{Concentration of arsenic or antimony (µg/ml)}}{\text{Mass of sample taken (g)}} \times 50$$

15.1.8 *Determination of Mercury by Atomic Absorption Cold Vapour Technique*

15.1.8.0 *Principle*

The sample is ashed by heating under reflux with sulphuric and nitric acids. The oxidation is completed by addition of potassium permanganate solution. After successive additions of hydroxylamine hydrochloride solution and stannous chloride solution, the mercury content is measured by cold vapour atomic absorption spectrometry

15.1.8.1 *Special reagents*

a) *Nitric acid* — sp gr 1.40;

b) *Sulphuric acid* — sp gr 1.84,

c) *Sulphuric acid* — approximately 3.5 M. Prepare by diluting 1 volume of concentrated sulphuric acid (b), with 4 volumes of water;

d) *Sulphuric acid* — approximately 1 M Prepare by diluting 1 volume of 3.5 M sulphuric acid (c) with 2.5 volumes of water,

e) *Hydrochloric acid* — sp gr 1.18;

f) *Potassium permanganate solution* — 50.0 g/L,

g) *Hydroxylamine hydrochloride solution* — 10.0 g/L;

h) *Stannous chloride solution* — Prepare by dissolving 25.0 g of stannous chloride ($SnCl_2.2H_2O$), in 50 ml hydrochloric acid (e). Make up to 250 ml with water and bubble nitrogen through the solution. Store over a few granules of metallic tin;

j) *Chromic acid mixture* — Dissolve 4.0 g of potassium dichromate in 300 ml of 3.5 M sulphuric acid (c) and make up to 1 litre with water;

k) *Magnesium perchlorate* — in granular form for gas desiccation; and

m) *Mercuric chloride.*

15.1.8.2 *Standards*

Use commercially available standard solutions, or prepare the standards as follows:

a) *Mercuric chloride solution* — 0.5 mg Hg/ml. Weigh out, to the nearest 0.1 mg, 0.677 g of mercuric chloride. Dissolve in approximately 250 ml 3.5 M sulphuric acid in a 1-litre volumetric flask, add approximately 700 ml water and then potassium permanganate solution dropwise until a colouration persists. Make up to the mark with water and mix well. Renew this solution every three months.

b) *Mercuric chloride solution* — 0.02 µg Hg/ml. Dilute the standard mercuric chloride solution 0.5 mg Hg/ml [standard (A)] by a factor of 25 000 by successive dilution with sulphuric acid [special reagent (d)], for example, 10 ml made up to 250 ml twice followed by 10 ml made up to 400 ml. Before bringing up to the mark in the final dilution, add potassium permanganate solution [special reagent (f)] dropwise until a colouration persists. Renew this solution daily.

15.1.8.3 *Apparatus*

All the glassware must by cleaned with hot nitric acid [special reagent (a)] and washed thoroughly with water before use.

a) *Mineralization apparatus* — fitted with reflux condenser (*see* Fig. 5).

b) *Bubblers* — with a ground glass stopper fitted with two tubes to permit entrainment of the mercury vapour and with a calibration mark at the required volume for measurement.

The capacity of the bubbler and position of the mark depend on the atomic absorption spectrophotometer used

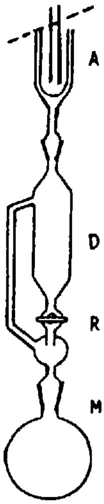


FIG 5 MINERALIZATION APPARATUS

Clean the bubbler successively with chromic acid mixture [special reagent (j)], tap water and double distilled water before use

c) *Water vapour absorption apparatus* — containing magnesium perchlorate [special reagent (k)]

d) *Atomic absorption spectrophotometer* — suitable for the cold vapour determination of mercury in open or closed circuit, with recorder

15.1.8.4 *Procedure*

Ashing — Weigh out, to the nearest 2 µg, approximately 0 5 g sample containing not more than 0 5 µg total mercury Introduce the sample into the receiver flask (*M*), and add a few glass beads Connect the receiver flask to the condensate reservoir (*D*) and close the stopcock (*R*)

Introduce into the reservoir 25 ml of nitric acid [special reagent (a)] followed by 10 ml sulphuric acid [special regent (b)]. Mount the turn on the condenser (*A*) open the stopcock carefully and allow small portions of the mixture of acids to run into the receiver flask Interrupt the flow of acids if the reaction becomes too vigorous

Empty the reservoir into the receiver flask, mix the contents of the latter well by careful shaking and leave the stopcock open

Heat the receiver flask carefully As soon as foaming has ceased, close the stopcock (*R*), continue heating and let the condensate collect in the reservoir

Discontinue heating when the contents of the receiver flask begin to char Allow a small portion of the condensate to run into the receiver flask, close the stopcock again and resume heating the receiver flask Repeat this procedure for as long as the contents display charring when heating

When charring has ceased, heat and add condensate as soon as white fumes appear Continue alternately heating and adding condensate for one hour Finally heat the contents of the flask to white fumes

Stop heating and allow to cool to approximately 40°C Open the stopcock and allow all the condensate to run into the receiver flask Wash the apparatus out from the top of the condenser with 5-10 ml of water, collect the washings in the receiver flask and disconnect it from the reservoir

Treatment of the solution — Introduce the potassium permanganate solution [special reagent (f)] dropwise into the receiver flask, with agitation, until a pink colouration persists Note the quantity of reagent (f) used (if this quantity exceeds 10 ml, repeat the procedure 'Ashing' as above)

Heat gently to boiling, then allow to cool

Pour the contents of the receiver flask into a bubbler, wash the receiver flask with water and add the washings to the contents of the bubbler

Measure the mercury content (*see* below) the same day as the treatment of the solution

Measurement of mercury content — Introduce 5 ml of hydroxylamine hydrochloride [special reagent (g)] into the bubbler and make up the mark either with double distilled water or with sulphuric acid [special reagent (d)] in the case of standard solutions Add 5 ml of stannous chloride solution [special reagent (g)], assemble the bubbler, connect it to the water vapour absorption apparatus and to the atomic absorption spectrophotometer Set the latter in operation

Mix the contents of the bubbler well by gently shaking, pass air or nitrogen through, measure and record Carry out measurements, as quickly as possible after the addition of stannous chloride If an open-circuit system is used, wait 30 sec before passing air or nitrogen

Calibration curve — Introduce respectively 2, 5, 10, 15 and 25 ml aliquots of the standard mercury solution [standard (b)] into bubblers and 25 ml sulphuric acid [special reagent (d)] into a sixth bubbler Add potassium permanganate solution [special reagent (f)] dropwise, with agitation, to each bubbler until a colouration persists

Measure the mercury content as described above

Plot the calibration curve with the measured absorption values as ordinates and the corresponding mercury contents in micrograms as abscissae The working standards contain 0, 0 04, 0 10, 0 20, 0 30 and 0 50 µg of mercury, respectively

Method of addition — The method of addition may be used if an open-circuit system is used.

Place one of the working standard solutions in a bubbler and add an aliquot portion of the sample solution obtained after treatment. The quantity of mercury in the bubbler must lie in the range in which the photometer gives a linear response, Measure the mercury content as described above. If necessary, carry out several such determinations, using different working standard solutions.

Blank determination — Carry out all the operations, from ashing to measurement, except for introduction of the sample. When treating the solution, add a quantity of potassium permanganate solution [special reagent (f)] equal to that used for the experimental sample.

15.1.8.5 *Calculation*

Read off from the calibration curve the quantities, in µg, of mercury corresponding to the measured absorption values.

Subtract the quantity of mercury found in the blank from that found in the sample:

$$\text{Hg in the sample, mg/kg} = \frac{\text{Net mass of mercury } (\mu g)}{\text{Sample mass (g)}}$$

15.2 Chemical Methods

15.2.0 Chemical methods have been given for determination of lead (Pb), arsenic (As), Copper (Cu) and chromium (Cr).

15.2.1 *Test for Lead*

15.2.1.1 *Apparatus*

a) *Digestion Funnel*

b) *Separatory Funnel*

15.2.1.2 *Reagents*

a) *Nitric acid* — 65 percent.

b) *Sulphuric acid* — sp gr 1.84.

c) *Ammonium acetate-citrate solution* — Dissolve 12.5 g of ammonium acetate and 12.5 g of ammonium citrate in water, add concentrated ammonia until the solution is alkaline to thymol blue paper and add water to 100 ml. Purify with 0.002 percent *m/v* solution of dithizone in carbon tetrachloride, and finally shake the solution with carbon tetrachloride to remove excess of dithizone.

d) *Ammonia solution* — 25 percent.

e) *Carbon tetrachloride*

f) *Ammonium hydroxide* — 0.2 N.

g) *Potassium cyanide* — 10 percent.

h) *Hydroxylamine hydrochloride solution* — 10 percent.

j) *Dithizone solution* — 0.1 percent (*m/v*), purified by the following procedure:

Dissolve the dithizone in chloroform and treat it with ammonia. Add mineral acid. Precipitate shall be pure dithizone. The aqueous ammoniacal solution obtained from chloroform solution of dithizone should be colourless; otherwise further purification as mentioned above should be carried out.

k) *Buffer* pH2 — Add 11.90 ml of 0.2 M hydrochloric acid and 88.10 ml of 0.2 M potassium chloride in a 200-ml volumetric flask and add water to volume.

15.2.1.3 *Procedure*

The limit test described for lead is designed to show if a sample contains more than 10 mg/kg or 20 mg/kg of lead. The sample is digested with nitric and sulphuric acids, and a clear solution of the digest is prepared.

Digestion — Weigh 1.0 g of sample for substances with 10 mg/kg limit or 0.5 g for those with 20 mg/kg limit. Place with 5 ml of water, 5 ml of 65 percent nitric acid and 5 ml of sulphuric acid in a digestion flask. Warm slightly. If foaming becomes excessive, add a little water. Evaporate the mixture. Maintain strongly oxidizing conditions in the flask during digestion by adding cautiously small quantities of nitric acid whenever the mixture begins to turn brown or dark. Continue digestion until organic matter is destroyed and sulphuric trioxide fumes are copiously evolved. The final solution should be colourless or at most slight straw colour.

To remove nitrosylsulphuric acid, after partial cooling transfer the residue to a dish, rinse with 25 ml of water, evaporate and heat again to fuming point. Add 25 ml of water and evaporate and heat again.

Solution of digest — Allow to cool, add 20 ml of ammonium acetate-citrate solution, and allow again to cool, neutralize to about *p*H 7 with 25 percent ammonia and boil if necessary to dissolve calcium sulphate. Cool the clear solution. Shake the solution with 10 ml of carbon tetrachloride and discard the lower layer.

Test with dithizone — To a 100-ml separatory funnel, add 10 ml of 0.2 N ammonium hydroxide, 2 ml of 10 percent potassium cyanide, 2 ml of 10 percent hydroxylamine hydrochloride solution and 2 ml of 20 mg per litre solution of dithizone in carbon tetrachloride. Shake the separatory funnel for a few minutes and discard the carbon tetrachloride layer. Add 2 ml of carbon tetrachloride, shake and discard again the carbon tetrachloride layer. Add the solution of digest to the separatory funnel. Again add 5 ml of dithizone solution in carbon tetrachloride and shake vigorously for a few minutes. The carbon tetrachloride layer becomes red according to the amount of lead present.

Treat simultaneously a standard solution containing 10 µg of lead in the same manner as the solution of the digest. Evaluate the quantity of lead in the digest by comparing the colour of the carbon tetrachloride layers.

Ensure that the red colour is due to lead by shaking the carbon tetrachloride layer obtained from the digest solution with 10 ml of a buffer *p*H2. The red colour will turn green if it is due to lead.

15.2.2 *Test for Arsenic*

15.2.2.0 Arsenic may be tested by either the method given below for routine purposes or the modified Gutzeit method as given in IS 2088 : 1983.

15.2.2.1 *Apparatus*

a) *Distillation appartus* — as shown in Fig. 6.

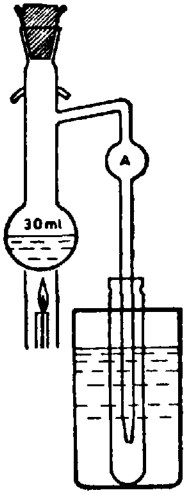


FIG. 6 DISTILLATION APPARATUS

b) *Conical flask* — 25 ml close, as shown in Fig. 7.

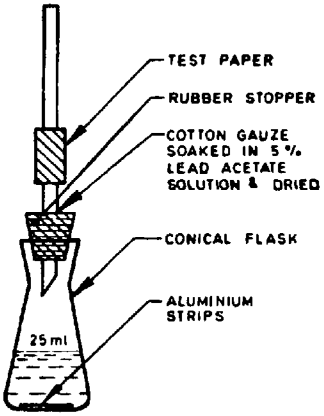


FIG. 7 CONICAL FLASK

15.2.2.2 *Reagents*

a) *Sulphuric acid* — sp gr 1.84.

b) *Potassium permanganate solution* — 0.1 N.

c) *Ferrous sulphate* — freshly powdered.

d) *Hydrochloric acid* — 38 percent.

e) *Potassium bromide solution* — 20 percent.

f) *Aluminium strips* — 8 mm × 8 mm × 1 mm.

g) *Tin chloride solution* — 2 percent tin chloride in 10 percent hydrochloric acid.

h) *Test paper* — Soak strips of filter paper in saturated ethanolic solution of mercuric bromide and allow to dry.

15.2.2.3 *Procedure*

The limit test prescribed for arsenic shows whether a sample contains more than 3 mg/kg of arsenic.

Digestion of 1 g sample and removal of nitrosylsulphuric acid as described in para 3 of **15.2.1.3**. Allow the digest, with 5 ml sulphuric acid, to cool.

Distillation (according to Snyder) — Pour the digest into the distillation flask (Fig. 6). Rinse the dish twice using each time 2.5 ml of water and add the water to the digest. Cool the solution. Add some drops of potassium permanganate solution until the red colour persists. Add 0.250 g of freshly powdered ferrous sulphate, 2.5 ml of 38 percent hydrochloric acid and 0.1 ml of potassium bromide solution.

Close the flask; place a reagent tube containing 8 ml of water in a beaker with water as shown in Fig. 6.

Heat with microburner. In the beginning a slow stream of air bubbles appear, followed after 3 to 5 minutes by hydrochloric acid gas. Heat then a little stronger so that the solution boils. After bulb *A* has become very hot, boil for 40 seconds. Thereupon lower the reagent tube and remove the flame. The hydrochloric acid content of the distillate shall be 9 to 10 percent only then has arsenic been completely distilled over; moreover this acid concentration is required for the test given below:

Test with mercury bromide paper (Mayercon - Bergeret) — The distillate should be transferred to a conical flask of 25 ml capacity. The flask should be closed with the device as shown in Fig. 7 containing a small disk of test paper. Add 3 pieces of aluminium strips, 1 ml of tin chloride solution and immediately close the flask with the stopper. Allow the flask to stand in a water-bath of 25° to 30°C for 50 to 60 minutes. At the same time carry out a parallel test using a solution of 2, 4, 6 or 8 µg of arsenic in 10 ml of 10 percent hydrochloric acid in place of the test sample. Compare the colour of the test paper for evaluating arsenic content of the sample.

15.2.3 *Determination of Copper*

15.2.3.1 *Reagents*

a) *Citric acid* — solid.

b) *Ammonium hydroxide solution* — sp gr 0.92 (not less than 27 percent ammonia).

c) *Concentrated hydrochloric acid*

d) *Dithizone (diphenyl thiocarbazone) solution* — 0.1 percent (*m*/*v*) in chloroform.

e) *Concentrated nitric acid*

f) *Concentrated sulphuric acid*

g) *Citric acid solution* — 5 percent (*m*/*v*), aqueous.

h) *Gum arabic solution* — one percent.

j) *Sodium diethyl dithiocarbamate solution* — 0.2 percent (*m*/*v*), aqueous, freshly prepared.

k) *Standard strong solution of copper* — Dissolve 0. 392 5 g of pure crystallized copper sulphate ($CuSO_4$, $5H_2O$) in water and make up the volume to 100 ml. This solution contains one milligram of copper per millilitre.

m) *Standard dilute solution of copper* — Dilute one millilitre of the standard strong solution of copper to 100 ml in a graduated flask. One millilitre of this solution contains 0.01 mg of copper.

15.2.3.2 *Procedure*

Take a suitable aliquot of the test solution prepared as described above and add 2 g of citric acid. Neutralize exactly with the ammonium hydroxide solution using a piece of litmus paper and acidify with one millilitre of concentrated hydrochloric acid. Cool and transfer to a separating funnel. The total volume of the solution should be about 100 ml.

Extract the copper by shaking with three successive portions of 5 ml of the solution of dithizone, shaking thoroughly for a minute for each extraction. Separate the dithizone layers and wash the combined dithizone extracts with about 10 ml of water. Transfer the dithizone extract to a tube of heat-resistant glass and evaporate the chloroform on a waterbath.

Heat the copper-dithizone residue in the test tube with one millilitre of concentrated sulphuric acid and a little of nitric acid until all organic matter is destroyed. Add 5 ml of water and re-heat to fuming stage. Cool, dilute with water and transfer the whole of the solution or a measured volume of the solution, depending upon the amount of copper present, to a Nessler cylinder. Add one millilitre of the citric acid solution and 4 ml of the ammonium hydroxide solution followed by 5 ml of the gum arabic solution and make up the volume to 50 ml with water. Add 5 ml of the sodium diethl dithiocarbamate solution and match the colour by adding the standard dilute solution of copper [reagent (m)] to a control cylinder containing the same quantities of reagents as present in the test solution. Calculate the copper content of the material in parts per million from the known volume of the standard dilute solution of copper required for matching.

15.2.4 *Determination of Chromium*

15.2.4.1 *Reagents*

a) *Magnesium nitrate solution* — 25 percent (*m*/*v*).

b) *Strong sulphuric acid solution* — 4 N.

c) *Potassium permanganate solution* — 0.1 N.

d) *Sodium azide solution* — 5 percent (*m*/*v*).

e) *Sodium dihydrophosphate* — 4 M.

f) *Diphenylcarbazide solution* — Dissolve 125 mg of diphenylcarbazide $[C_6H_5.NH.NH)_2CO]$ in a mixture of 25 ml of acetone and 25 ml of water. This should be prepared immediately before use.

g) *Standard chromium solution* — Dissolve 0.056 6 g of potassium dichromate ($K_2Cr_2O_7$) in one litre of water. One millilitre of this solution contains 0.02 mg of chromium.

h) *Sucrose*

15.2.4.2 *Procedure*

Weigh 1.0 g of the sample into a quartz dish. Char the material, raising the temperature slowly. Allow to cool, add 10 ml of the magnesium nitrate solution and evaporate, heating slowly until no more nitrous vapour evolves. Heat the material in an oven at 600 ± 20°C until all black particles have disappeared (30 to 60 minutes). Dissolve the residue by adding 10 ml of the strong sulpuric acid solution and 20 ml of water. Heat on a water-bath for about 5 minutes.

Add 0.5 ml of the potassium permanganate solution, cover with a watch-glass and heat on a water-bath for about 20 minutes. Add more potassium permanganate, if the solution decolourizes. Add sodium azide solution, one drop every 10 seconds, until the excess potassium permanganate has been removed. (Avoid excess of sodium azide, 2 drops are usually sufficient.) Cool the solution in running water and filter, if manganese dioxide is evident. Transfer the solution to a 50-ml graduated flask. Add 2.5 ml of sodium dihydrophosphate and 2 ml of diphenylcarbazide solution and fill to the mark with water. Measure the extinction at 540 nm, 30 minutes after adding the diphenylcarbazide solution. A blank with the last two reagents (sodium dihydrophosphate and diphenyl-carbazide solutions) should show no colour or only a slight purple colour. At the same time, run a parallel test with 1.00 ml of the standard chromium solution and a few milligrams of sucrose placed in a second quartz dish. Treat the mixture exactly as the sample and measure the extinction at the same wavelength.

Calculate the chromium content of the sample from the two extinction values observed.

16 DETERMINATION OF HEAVY METALS

16.1 Reagents

16.1.1 *Ammonia Solution* — Dilute 400 ml of ammonium hydroxide (28 percent) to 1 000 ml with water.

16.1.2 *Hydrochloric Acid* — 10 percent.

16.1.3 *Lead Nitrate Stock Solution*

Dissolve 159.8 mg of lead nitrate in 100 ml of water containing 1 ml of nitric acid. Dilute with water to 1 000 ml and mix. Prepare and store the solution in lead-free glass containers.

16.1.4 *Standard Lead Solution*

Dilute 10 ml of lead nitrate stock solution, accurately measured, with water to 100 ml. Each millilitre of the solution so prepared contains the equivalent of 10 mcg of lead ion (Pb). Prepare the solution on the day of use.

16.1.5 *Nitric Acid* — 10 percent.

16.1.6 *Sulphuric Acid* — 94.5 to 95.5 percent.

16.1.7 *Hydrogen Sulphide*

A saturated solution of hydrogen sulphide made by passing hydrogen sulphide gas through cold water.

16.2 Procedure

16.2.1 *Solution A*

Take 1.25 ml of the standard lead solution in 50-ml Nessler tube and add 22.5 ml of water. Adjust the *p*H to between 3.0 to 4.0 by addition of acetic acid or ammonia solution. Dilute with water to 40 ml and mix.

16.2.2 *Solution B*

Place 500 mg of the sample, accurately weighed, in a suitable crucible, add sufficient sulphuric acid to wet the sample, and carefully ignite at a low temperature until thoroughly charred, covering the crucible loosely with a suitable lid during the ignition. After the substance is thoroughly carbonized, add 2 ml of nitric acid and 5 drops of sulphuric acid, and cautiously heat until white fumes are evolved, then ignite, preferably in muffle furnace, at 500 to 600°C until the carbon is completely burned off. Cool and add 4 ml of dilute hydrochloric acid, cover and digest on a steam-bath to dryness. Moisten the residue with one drop of hydrochloric acid, add 10 ml of hot water and digest for 2 minutes. Add dropwise ammonia solution until the solution is just alkaline to litmus paper, dilute with water to 25 ml and adjust the *p*H to between 3.0 and 4.0 (*p*H indicator paper) by the addition of diluted acetic acid. Filter if necessary, wash the crucible and the filter with 10 ml of water. Transfer to a 50-ml Nessler tube. Dilute the combined filtrate and washing with water to 40 ml and mix.

16.2.3 To each tube add 10 ml of freshly prepared hydrogen sulphide, mix and allow to stand for 5 minutes and view over a white surface. The colour of solution B shall not be darker than of solution A.

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Headquarters:

Manak Bhavan, 9 Bahadur Shah Zafar Marg, New Delhi 110002
Telephones 2323 0131, 2323 3375, 2323 9402 *website* . www bis.org.in

Regional Offices:		Telephones
Central	Manak Bhavan, 9 Bahadur Shah Zafar Marg NEW DELHI 110002	2323 7617 2323 3841
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